HIV-1 gp120 ANTIGEN CAPTURE ASSAY

Enzyme Immunoassay for the detection of Human Immunodeficiency Virus Type 1 (HIV-1) gp120 in tissue culture media.

Catalog #5429
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INTRODUCTION

The Human Immunodeficiency Virus Type 1 (HIV-1) is the etiological agent of Acquired Immunodeficiency Syndrome (AIDS) in humans. The envelope of HIV-1 is made up of external envelope glycoprotein gp120 and transmembrane glycoprotein gp41. Unlike the core antigen p24, the gp120 is highly variable among different isolates of HIV-1. Thus, the gp120 exhibits a number of highly type specific antigenic determinants. The HIV-1 gp120 Antigen Capture Assay is a double antibody sandwich enzyme immunoassay that is used to calculate the concentration of Clade B HIV-1 gp120 in tissue culture samples. The assay has a linear range of 62.5 to 2,000 pg/ml. The assay is applicable for only Clade B isolates and detects the X4 or R5 isolates with comparable sensitivity. The sensitivity for isolates of other clades may be greatly reduced.

ASSAY OVERVIEW

Test Samples are mixed with Disruption Buffer to inactivate virus and to release gp120 into solution. The microtiter wells of a 96-well plate are coated with two murine monoclonal antibodies that react with unique epitopes on HIV-1 gp120. When HIV-1 gp120 Standard Solutions or tissue culture Test Samples are added to the wells, an immune complex forms with the plate-bound antibodies and the gp120 in solution. Unbound materials are then thoroughly washed away. Conjugate Solution, containing peroxidase-conjugated human anti-gp120 polyclonal antibodies, is then added. The conjugated antibodies complex with the captured gp120. After washing the wells to remove the unbound conjugated antibodies, Peroxidase Substrate is added to the wells. The enzyme-substrate reaction results in a blue color change. Upon adding Stop Solution, the blue color changes to yellow, and the absorbance is measured at 450 nm. There is a linear relationship between the absorbance at 450 nm and the amount of HIV-1 gp120 bound to the well. The concentration of HIV-1 gp120 in Test Samples can be determined from linear regression analysis of the standard curve.

PRODUCT WARRANTY

This product is for research use only and should not be used for clinical diagnostic purposes. ABL guarantees the quality and performance of all products used before the expiration date printed on the label. If a product is used according to manufacturer’s instructions and fails to perform as described in the manual, please contact ABL to speak with a technical representative.
## ASSAY COMPONENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microelisa Plate</td>
<td>1 plate</td>
</tr>
<tr>
<td>Disruption Buffer</td>
<td>1 bottle of 10 ml</td>
</tr>
<tr>
<td>Conjugate Solution</td>
<td>1 bottle of 12 ml</td>
</tr>
<tr>
<td>Peroxidase Substrate</td>
<td>1 bottle of 12 ml</td>
</tr>
<tr>
<td>HIV-1 gp120 Standard</td>
<td>2 vials</td>
</tr>
<tr>
<td>Wash Buffer (20X)</td>
<td>2 bottles of 25 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 bottle of 12 ml</td>
</tr>
<tr>
<td>Plate Sealers</td>
<td>5 adhesive sheets</td>
</tr>
</tbody>
</table>

- **Microelisa Plate**: 96 well plate coated with murine monoclonal antibodies to HIV-1 gp120. Plate is contained in a resealable foil pouch with desiccant.
- **Disruption Buffer**: Contains Triton® X-100 detergent and phosphate buffer.
- **Conjugate Solution**: Contains horseradish peroxidase-labeled, immunoaffinity purified, human antibodies to HIV-1 gp120.
- **Peroxidase Substrate**: Contains hydrogen peroxide and tetramethylbenzidine in an acidic buffer.
- **HIV-1 gp120 Standard**: Contains lyophilized HIV-1 gp120 at 10 ng/ml when reconstituted.
- **Wash Buffer (20X)**: Contains Phosphate Buffered Saline / Tween 20® concentrate.
- **Stop Solution**: Contains 2N sulfuric Acid. **Warning – sulfuric acid is corrosive and can cause severe burns to skin and eyes.**
- **Plate Sealers**: Adhesive sheets.

**Triton® X-100** is a registered trademark of The Dow Chemical Company. Tween 20® is a registered trademark of ICI Americas.

**Store all kit components at 2-8ºC. Do not freeze reagents**

## ADDITIONAL REQUIRED MATERIALS

- Distilled water
- Complete tissue culture medium, containing 10% fetal bovine serum (FBS)
- Absorbent paper (paper towels)
- Timer
- V-bottomed reagent reservoirs
- Multichannel or single channel pipettes and pipette tips
- Incubator, 37º ± 0.5ºC
- Microelisa plate washing system
- Microelisa plate reader (single wavelength 450 nm ± 5 nm)
ASSAY PROCEDURE

Preliminary Notes

1. The HIV-1 gp120 Antigen Capture Assay is for research use only and is not intended for diagnostic or clinical use.

2. For consistent results, bring all components and samples to room temperature (19-23°C) before use. Return the reagents to 2-8°C after use.

3. Always bring the foil pouch containing the Microelisa Plate to room temperature (19-23°C) before opening. After opening, unused microelisa strips can be stored for up to 2 months at 2-8°C, provided that the foil pack is resealed and the desiccant is not removed.

4. All reagents can be used only once.

Cautions

1. Handle all reagents and samples as if capable of transmitting disease. The Conjugate Solution contains human derived material, and the HIV-1 gp120 Standard contains virus derived material. Although these reagents have been inactivated, there is no absolute assurance that such products cannot transmit infection. We recommend that all materials, samples and reagents be handled in accordance with the Occupational Safety and Health Administration (OSHA) and the Centers for Disease Controls and Prevention (CDC) guidelines for working with HIV. Always follow Good Laboratory Practice (GLP) guidelines.

2. Always wear personal protective equipment, including gloves and lab coats, when handling kit reagents and samples.

3. Dispose of all materials, samples and reagents used in this assay as hazardous waste.

4. The Stop Solution contains 2N sulfuric acid, which can cause severe burns to the skin and eyes. Because sulfuric acid is corrosive, waste liquids containing sulfuric acid should be neutralized before disposal.

5. The Stop Solution should never come in contact with Sodium Hypochlorite (bleach).
**Sample Preparation**

1. Tissue culture Test Samples should be free of particulate matter. Centrifuge Test Samples to remove cells and cell debris before use.

2. Test Samples must be free of microbial contamination.

3. Test Samples can be stored at –60° to -80°C before testing. However, avoid many freeze-thaw cycles, as they invalidate results.

4. Test Samples may require dilution in complete tissue culture media (+ 10% FBS) to be within the range of the assay.

**HIV-1 gp120 Standard Reconstitution**

1. Warm 1 vial of HIV-1 gp120 Standard to room temperature (19-23° C).

2. Reconstitute the HIV-1 gp120 Standard in 500 µl complete tissue culture media (containing 10% FBS). Gently swirl contents for 10 seconds and invert 5 times. Set at room temperature for 5 minutes and invert again 5 times. The concentration of HIV-1 gp120 is 10 ng/ml.

3. The reconstituted standard is stable for 1 week when stored at 2-8°C and is stable for 2 months when stored frozen at –60° to -80°C. Reconstituted standard is stable after a single freeze-thaw. Avoid multiple freeze-thaw cycles.

**Wash Procedure**

1. If salt crystals are evident in the Wash Buffer (20 X), incubate at 37°C until crystals dissolve.

2. Dilute 25 ml Wash Buffer (20X) in 475 ml distilled water. Mix thoroughly. Diluted Wash Buffer will remain stable for 1 month at 4°C.

3. If using a plate washing system, aspirate the well contents into a waste flask. Fill the wells with 300 µl diluted Wash Buffer (19-23°C), soak for 15 seconds, and then aspirate. Repeat wash procedure for a total of four washes.

4. After the last aspiration, invert the Microelisa Plate and tap firmly on absorbent paper (paper towel). Be careful not to dislodge any strips while tapping.

Alternatively: In absence of a plate washing system, drain plate and tap on absorbent paper (paper towel), then add diluted Wash Buffer. Following each 15-second soak, drain and tap dry on clean absorbent paper.
Test Procedure

1. Add 25 µl of Disruption Buffer to each well of the Microelisa Plate to be used in the assay.

2. Dilute the reconstituted 10 ng/ml HIV-1 gp120 Standard in complete tissue culture media (containing 10% FBS) by the following method:

<table>
<thead>
<tr>
<th>HIV-1 gp120 Standard Volume</th>
<th>Complete Tissue Culture Media Volume</th>
<th>Final Diluted HIV-1 gp120 Standard Concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µl of 10 ng/ml</td>
<td>+ 400 µl</td>
<td>= 2,000</td>
</tr>
<tr>
<td>250 µl of 2,000 pg/ml</td>
<td>+ 250 µl</td>
<td>= 1,000</td>
</tr>
<tr>
<td>250 µl of 1,000 pg/ml</td>
<td>+ 250 µl</td>
<td>= 500</td>
</tr>
<tr>
<td>250 µl of 500 pg/ml</td>
<td>+ 250 µl</td>
<td>= 250</td>
</tr>
<tr>
<td>250 µl of 250 pg/ml</td>
<td>+ 250 µl</td>
<td>= 125</td>
</tr>
<tr>
<td>250 µl of 125 pg/ml</td>
<td>+ 250 µl</td>
<td>= 62.5</td>
</tr>
</tbody>
</table>

3. Add 100 µl of each diluted HIV-1 gp120 Standard to microelisa wells containing Disruption Buffer, in duplicate.

4. To serve as Negative Controls, add 100 µl of Complete Tissue Culture Media (containing 10% FBS) to wells containing Disruption Buffer, in quadruplicate.

5. Add 100 µl of the prepared Test Samples to microelisa wells containing Disruption Buffer. It is recommended that these be performed in duplicate. It may be necessary to add several dilutions of the Test Samples to ensure results will be within the assay range.

6. Gently tap the side of the plate to mix, cover with a Plate Sealer and incubate at 37 ± 0.5°C for 60 ± 2 minutes.

7. Wash Microelisa Plate according to the previously stated Wash Procedure.

8. Add 100 µl of Conjugate Solution to each well.

9. Cover wells with a fresh Plate Sealer and incubate at 37 ± 0.5°C for 60 ± 2 minutes.

10. Wash Microelisa Plate according to the previously stated Wash Procedure.

11. Add 100 µl of Peroxidase Substrate to each well.

12. Incubate plate uncovered for 30 ± 1 minute at room temperature (19-23°C).
13. In the same order that the Peroxidase Substrate was added, add 100 µl of Stop Solution to each well. **Warning** – **Stop Solution contains 2N sulfuric acid, which is corrosive and can cause severe burns to skin and eyes.**

14. Read the plate absorbance at 450 nm in a Microelisa Plate Reader within 20 minutes.

**Results**

**Qualification of Negative Controls Values**
Negative Control absorbance values over 0.200 are not acceptable. If two or more values are above 0.200, then the run is invalid. Check washing procedure, incubation times and temperatures and component expiration dates.

**Qualification of HIV-1 gp120 Standards Values**
The absorbance values of the 2,000 pg/ml HIV-1 gp120 Standard should be >1.2 and <2.2. If values are not within this range, then the run is invalid. Check washing procedure, incubation times and temperatures and component expiration dates.

**Qualification of Test Sample Values**
To be considered valid, Test Sample absorbance values should be between those of the 62.5 pg/ml and the 2,000 pg/ml HIV-1 gp120 Standards. If a Test Sample absorbance value is below the 62.5 pg/ml HIV-1 gp120 Standard value, then the gp120 concentration is below the sensitivity of the assay. If a Test Sample absorbance value is above the 2,000 pg/ml HIV-1 gp120 Standard value, then the run must be repeated with a more diluted Test Sample to be within the assay’s effective range.

**Calculation of Test Sample HIV-1 gp120 Concentration**
1. Calculate the mean absorbance for each HIV-1 gp120 Standard, Negative Control, and Test Sample. To subtract the background, subtract the mean absorbance of the Negative Controls from the mean absorbance of the HIV-1 gp120 Standards and Test Samples.

2. Determine the HIV-1 gp120 concentration of each Test Sample by interpolating from a standard curve or by using linear regression analysis.
TYPICAL STANDARD CURVE

![Graph showing the typical standard curve for HIV-1 gp120 antigen capture assay.](image)

<table>
<thead>
<tr>
<th>STANDARD (pg/ml)</th>
<th>A450 1</th>
<th>A450 2</th>
<th>A450 MEAN</th>
<th>A450 BACKGROUND</th>
<th>pg/ml</th>
<th>A450 BACKGROUND COMPUTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 gp120</td>
<td>2000</td>
<td>1.768</td>
<td>1.822</td>
<td>1.795</td>
<td>1.727</td>
<td>2005</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.909</td>
<td>0.929</td>
<td>0.919</td>
<td>0.851</td>
<td>985</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.502</td>
<td>0.512</td>
<td>0.507</td>
<td>0.439</td>
<td>506</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.289</td>
<td>0.297</td>
<td>0.293</td>
<td>0.225</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>0.178</td>
<td>0.184</td>
<td>0.181</td>
<td>0.113</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>0.123</td>
<td>0.124</td>
<td>0.124</td>
<td>0.056</td>
<td>59.0</td>
</tr>
<tr>
<td>NEGATIVE CONTROL (BACKGROUND)</td>
<td>0</td>
<td>0.066</td>
<td>0.066</td>
<td>0.068</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.071</td>
<td>0.068</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Regression Output:

- Constant: 0.005
- Std Err of Y Est: 0.008
- R Squared: 1.000
- No. of Observations: 6.000
- Degrees of Freedom: 4.000

X Coefficient(s): 0.001
Std Err of Coef.: 0.00000
TROUBLESHOOTING GUIDE

Weak signal
Check incubation times and temperatures. If reagents are not allowed to reach room temperature prior to use or if the room temperature is cooler than acceptable range (19-23°C), the absorbance values may be unacceptably low. Make sure reagents have been warmed to room temperature prior to use.

If there are multiple plates in the same 37°C incubator, the plate may require more time to reach 37°C. In such instances, incubation times may be increased up to an additional one half hour.

No signal
Check procedures used. Make sure proper diluent is used for samples and standards.

Check pH of wash buffer. Acceptable range is pH 7.4 ± 0.3.

High background (two or more Negative Controls are above 0.200 absorbance)
Check washing procedure, incubation times and temperatures and component expiration dates. Be certain the medium used to dilute samples and standards is fresh.

Plate washers may vary in their efficiency to aspirate liquid from wells. Trace amounts of Conjugate Solution may react with the Peroxidase Substrate causing high background. Adding additional washing steps may reduce background.

Sample values are higher than the 2,000 pg/ml Standard
Dilute samples further so they are within the effective range of the assay.

Salt crystals in Disruption or Wash Buffer (20 X)
Reagents may have been exposed to temperatures below the optimum range (2-8°C). Warm to 37°C until crystals dissolve.

TECHNICAL ASSISTANCE

We value our customer’s feedback as it allows us to keep improving our products. We encourage you to contact ABL if you have any questions or concerns: 800-225-5600 or info@ablinc.com.